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13. ABSTRACT *(Maximum 200 words)*

We have developed a microfabricated system for sorting cells for 'directed evolution.' Our microfabricated fluorescence-activated cell sorter (FACS) can be used for the sorting of various biological entities, ranging from E. coli cells to single molecules of DNA. The actual cell-sorting is performed on a disposable "micro-machined chip" with a detection volume of ~250 femtoliter. Our intent is to construct a cell-sorter that is smaller in size, higher in sensitivity and lower in cost than the conventional electrostatic FACS apparatus. We hope to apply this technology to various chemical and biological studies, including molecular evolution of technologically-useful enzymes. This project is a collaboration with Professor Stephen Quake in Applied Physics.



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# METAL-MEDIATED TWO-DIMENSIONAL PROTEIN CRYSTALLIZATION

FINAL PROGRESS REPORT

FRANCES H. ARNOLD

1 September 1995 – 31 August 1999

U.S. ARMY RESEARCH OFFICE

GRANT NUMBER DAAH04-95-1-0613

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## Problem studied

The research funded under this grant originally focused on developing a general system for 2-D protein crystallization on lipid monolayers. Membrane targeting of soluble ligands, accompanied by assembly of membrane components or the ligands themselves into superstructures, underlies a variety of important biological processes. Protein or lipid components provide the interactions required for targeting and specific orientation of bound molecules; the membrane's fluidity allows reorganization and sampling of intermolecular contacts required for assembly into more complex structures. A goal of our research was to devise simple and robust systems that can reproduce in synthetic membranes some of the features of biological targeting and assembly, processes that would be of considerable use in the development of biosensors, biomolecular devices, or synthesis of novel organic and inorganic materials.

About half way into the grant period we decided to change the direction of the project. During this time, I decided to focus on molecular evolution, and one of the most exciting opportunities in this area was developing new high-throughput screening technologies. Therefore we decided to use the remaining grant period to develope a microfabricated, microfluidic system for sorting cells and other biological entities used for in vitro evolution. Our microfabricated fluorescence-activated cell sorter (μ-FACS) was to be used for the sorting of various biological entities, ranging from E. coli cells to single molecules of DNA. This project was a collaboration with Professor Stephen Quake and Professor Axel Scherer in Applied Physics at Caltech.

## Summary of key results

We succeeded in forming 2-dimensional crystals of streptavidin (SA) on monolayers of synthetic lipids containing chelated metal ions (copper) (DOIDA-Cu and PSIDA-Cu) that bind histidines on the protein surface. The crystals were studied by Brewster angle microscopy (at the University of Washington), fluorescence microscopy and transmission electron microscopy (at Stanford University). Crystals grown on the less-fluid PSIDA monolayer are typically smaller in size than those grown on DOIDA (25 vs 50 μm), while the morphologies are very similar. At the onset of protein crystallization (surface pressure of 3 mN/m), Cu-PSIDA and Co-DOIDA monolayers are nearly equally compressible. After crystallization, PSIDA monolayers are only half as compressible as the DOIDA monolayers. Lateral mobility is believed to aid crystal growth. Crystallization on both monolayers has been shown to be reversible, by addition of EDTA or imidazole.

The effect of decreasing the density of Cu-PSIDA on SA self-assembly was also studied, by mixing Cu-PSIDA with an inert matrix lipid, SOPC. All Cu-PSIDA/SOPC mixtures containing more than 40% Cu-PSIDA will support the formation of 2D SA crystals large enough to be observed by fluorescence microscopy. Crystallinity was

confirmed by fluorescence polarization and by transmission electron microscopy. The lattice parameters obtained are similar to those of 2D SA crystals grown on biotinylated lipids.

Changes in the monolayer during crystallization studied using the fluorescence properties of the Cu-PSIDA. Domains containing bound protein exhibit quenching of the pyrene excimer fluorescence, which may reflect reduced mobility of the lipid layer directly above the crystalline protein domains. Alternatively, quenching could reflect penetration of the monolayer by the protein or a local increase in monolayer area above the crystallized protein.

Professor Deborah Leckband of the University of Illinois used the Cu-IDA as part of their effort to determine the electrostatic properties of different surface regions of the protein cytochrome b5 using the surface forces apparatus. Densely packed monolayers of cytochrome b5 with a 6-histidine tag at the N-terminus of the protein are anchored specifically and in a controlled orientation to mixed lipid monolayers containing Cu-IDA. The electrostatic properties of the outer surface (distal to the lipid substrate) of the cyt b5 were probed by direct measurements with the surface forces apparatus. The electroneutrality of the Cu-IDA complex enabled them to measure the electrostatic properties of the protein monolayer absent any electrostatic interference from the underlying substrate. These measurements were then compared with the electrostatic surface potentials of cytochrome b5 monolayers covalently bound in different orientations to lipid monolayers. In the latter cases, sulfhydryl reactive lipids were used to anchor the proteins. With this general approach, differences in the protein surface electrostatic properties were detected, which are attributed to the different electrostatic properties of the probed surface regions. In this manner, we are obtaining a low resolution electrostatic potential map of the cytochrome b5 surface. This group also determined the orientation of the 6-his b5 on the Cu-IDA monolayers by linear dichroism and direct force measurements.

In the final year of the project we successfully constructed a microfabricated, microfluidic fluorescence-activated cell sorter that is smaller in size, higher in sensitivity and lower in cost than conventional FACS. The goal is to apply this technology to various chemical and biological studies, including molecular evolution. The actual sorting is performed on a disposable microfabricated chip with a detection volume of ~250 femtoliters (Fig. 1). We have used the μ-FACS chips to obtain substantial enrichment of micron-sized fluorescent bead populations of differing colors (80-95X in a single pass). Furthermore, we have separated *E. coli* expressing green fluorescent protein from a background of non-fluorescent cells, and shown that the bacteria are viable after extraction from the sorting device. These sorters can function as stand-alone devices or as a component of an integrated microanalytical chip.

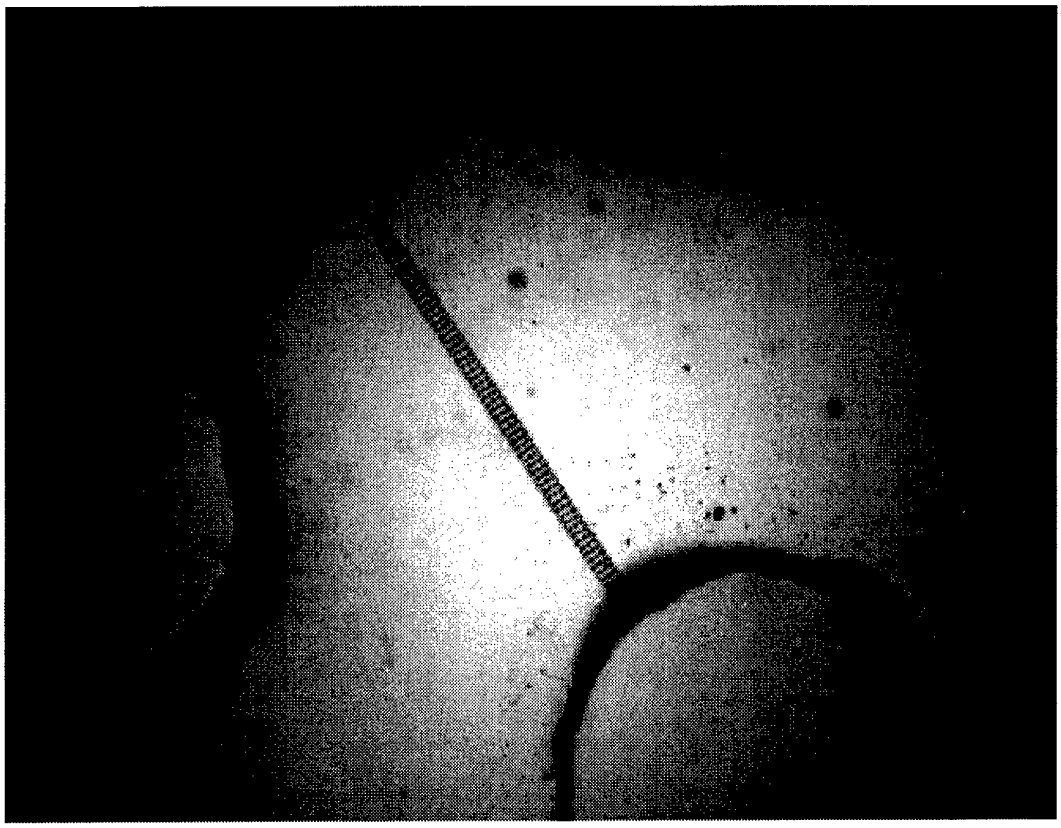

Figure 1. Optical micrograph of the μ-FACS device.

## Publications


"Two-Dimensional Protein Crystallization via Metal-Ion Coordination by Naturally-Occurring Surface Histidines," W. Frey, W. R. Schief, Jr., D. W. Pack, C-T Chen, A. Chilkoti, P. Stayton, V. Vogel and F. H. Arnold, *Proc. Natl. Acad. Sci. USA*, **93**, 4937-4941 (1996).

"Fluorescent Signaling of Ligand Binding and Assembly in Metal-Chelating Lipid Membranes," K. M. Maloney, D. R. Shnek, D. Y. Sasaki and F. H. Arnold, *Chemistry & Biology*, **3**, 185-192 (1996).

"Ligand-Induced Reorganization and Assembly in Synthetic Lipid Membranes," D.W. Pack, K. Ng., K. M. Maloney, F. H. Arnold, *Supramolecular Science*, **4**, 3-10 (1997).

"Langmuir Monolayer Characterization of Metal Chelating Lipids for Protein Targeting to Membranes," D. W. Pack and F. H. Arnold, *Chemistry & Physics of Lipids*, **86**, 135-152 (1997).

"A Metal Chelating Lipid for 2D Protein Crystallization via Coordination of Surface Histidines," D. W. Pack, G. Chen, K. Maloney, C.-T. Chen and F. H. Arnold, *J. Am. Chem. Soc.*, **119**, 2479-2487 (1997).

"Protein Binding and Two-dimensional Crystallization at the Air-water Interface via Engineered Cu-IDA Chelator Lipids," K. M. Maloney, W. R. Schief, Jr., F. H. Arnold and V. Vogel, *Coord. Chem. Reviews*, **183**, 3-18 (1999).

"A Microfabricated Fluorescence-Activated Cell Sorter," A. Y. Fu, C. Spence, A. Scherer, F. H. Arnold and S. R. Quake, *Nature Biotechnology*, **17**, 1109-1111 (1999).

**Scientific personnel supported (in part) by this project**

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**Inventions**

Microfabricated Sorter for Biological and Chemical Materials